NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 448

TOXICOLOGY AND CARCINOGENESIS STUDIES OF ISOBUTYL NITRITE (CAS NO. 542-56-3) IN F344/N RATS AND B6C3F$_1$ MICE (INHALATION STUDIES)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Technical Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

These NTP Technical Reports are available for sale from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161 (703-487-4650). Single copies of this Technical Report are available without charge while supplies last from NTP Central Data Management, NIEHS, P.O. Box 12233, MD E1-02, Research Triangle Park, NC 27709 (919-541-3419). Listings of all published NTP reports and ongoing studies are also available from NTP Central Data Management. The Abstracts and other study information for 2-year studies are also available at the NTP's World Wide Web site: http://ntp-server.niehs.nih.gov.

NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF

ISOBUTYL NITRITE

(CAS NO. 542-56-3)

IN F344/N RATS AND B6C3F$_1$ MICE

(INHALATION STUDIES)

July 1996

NTP TR 448

NIH Publication No. 96-3364

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

CONTRIBUTORS

National Toxicology Program

Evaluated and interpreted results and reported findings

K.M. Abdo, Ph.D., Study Scientist
D.A. Bridge, B.S.
J.R. Bucher, Ph.D.
M.R. Elwell, D.V.M., Ph.D.
T.J. Goehl, Ph.D.
J.K. Haseman, Ph.D.
A. Radovsky, D.V.M., Ph.D.
G.N. Rao, D.V.M., Ph.D.
J.H. Roycroft, Ph.D.
R.C. Sills, D.V.M., Ph.D.
G.S. Travlos, D.V.M.
D.B. Walters, Ph.D.
K.L. Witt, M.S., Oak Ridge Associated Universities

IIT Research Institute

Conducted studies, evaluated pathology findings

C. Aranyi, B.S., Principal Investigator
J.D. Fenters, Ph.D.
D. Kirkpatrick, Ph.D.
W. O'Shea, M.S.
A. Snelson, Ph.D.

Experimental Pathology Laboratories, Inc.

Provided pathology quality assurance

J.F. Hardisty, D.V.M., Principal Investigator
E.T. Gaillard, D.V.M., M.S.

Dynamac Corporation

Prepared quality assurance audits

S. Brecher, Ph.D., Principal Investigator

Analytical Sciences, Inc.

Provided statistical analyses

R.W. Morris, M.S., Principal Investigator
N.G. Mintz, B.S.
S. Rosenblum, M.S.

NTP Pathology Working Group

Evaluated slides, prepared pathology report on rats (25 August 1992)

P.K. Hildebrandt, D.V.M., Chair
PATHCO, Inc.
J. Bond, D.V.M.
North Carolina State University
E.T. Gaillard, D.V.M., M.S.
Experimental Pathology Laboratories, Inc.
J.R. Hailey, D.V.M.
National Toxicology Program
J. Mahler, D.V.M.
National Toxicology Program
D. Meuten, D.V.M., Ph.D.
North Carolina State University
C.C. Shackelford, D.V.M., M.S., Ph.D.
National Toxicology Program

Evaluated slides, prepared pathology report on mice (22 October 1992)

P.K. Hildebrandt, D.V.M., Chair
PATHCO, Inc.
C. Botteron, D.V.M.
North Carolina State University
J. Everitt, D.V.M.
Chemical Industry Institute of Toxicology
E.T. Gaillard, D.V.M., M.S.
Experimental Pathology Laboratories, Inc.
J.R. Hailey, D.V.M.
National Toxicology Program
C.C. Shackelford, D.V.M., M.S., Ph.D.
National Toxicology Program
R.C. Sills, D.V.M., Ph.D.
National Toxicology Program

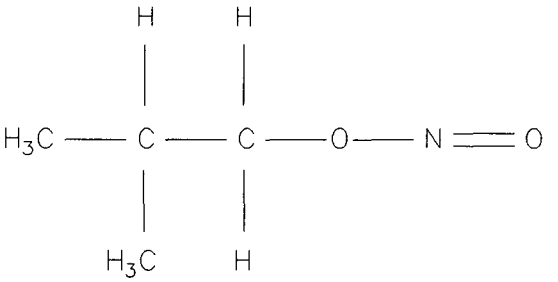
Biotechnical Services, Inc.

Prepared Technical Report

D.D. Lambright, Ph.D., Principal Investigator
C.M. Bailey, B.S. Pharm.
J.R. Beverly, B.A.
S.R. Gunnels, M.A.
T.A. King-Hunter, B.S.
T.L. Rhoades, B.S.

CONTENTS

ABSTRACT



ISOBUTYL NITRITE

CAS No. 542-56-3

Chemical Formula: $C_4H_9NO_2$ Molecular Weight: 103.12

Synonyms: IBN; iso-butyl nitrite; nitrous acid, isobutyl ester; nitrous acid, 2-methylpropyl ester

Isobutyl nitrite is used to a limited extent as an intermediate in the syntheses of aliphatic nitrites. It is also an ingredient of various incenses or room odorizers and is used as a euphoric. The chemical has also been used as a jet propellant and in the preparation of fuels. Isobutyl nitrite was nominated by the Consumer Product Safety Commission to the NTP for toxicology and carcinogenicity studies because of its possible contribution to the high incidence of Kaposi's sarcoma among male homosexual acquired immune deficiency syndrome patients and because of the lack of available data on the potential carcinogenicity of isobutyl nitrite. Male and female F344/N rats and B6C3F$_1$ mice were exposed to isobutyl nitrite (purity of 93% or greater) by inhalation for 16 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, cultured Chinese hamster ovary cells, *Drosophila melanogaster*, and mouse peripheral blood.

16-DAY STUDY IN RATS

Groups of five male and five female F344/N rats were exposed to 0, 100, 200, 400, 600, or 800 ppm (approximately 420, 840, 1,700, 2,500, or 3,300 mg/m^3) isobutyl nitrite by inhalation for 6 hours per day, 5 days per week for a total of 12 exposures during a 16-day period. All males and females exposed to 600 or 800 ppm and one 400 ppm female died on the first day of the study. Final mean body weights and mean body weight gains of 400 ppm males and females were significantly lower than those of the controls. Clinical findings observed in 400 ppm males and females included ocular discharge, lethargy, hunched posture, and rough coats. Absolute and relative lung weights of all exposed groups of males and of 200 and 400 ppm females were less than those of the controls. Chemical-related hyperplasia of the bronchial epithelium was observed in 200 and 400 ppm males and females and hyperplasia of the nasal turbinate epithelium was observed in rats exposed to 400 ppm or less. Hemosiderin pigmentation was observed in the spleen of 200 and 400 ppm males and females and bone marrow hematopoietic hyperplasia was observed in rats exposed to 400 ppm or less.

16-DAY STUDY IN MICE

Groups of five male and five female B6C3F$_1$ mice were exposed to 0, 100, 200, 400, 600, or 800 ppm (approximately 420, 840, 1,700, 2,500, or 3,300 mg/m^3) isobutyl nitrite by inhalation for

6 hours per day, 5 days per week for a total of 12 exposures during a 16-day period. Three males and four females exposed to 800 ppm died before the end of the study. Final mean body weights and mean body weight gains of 600 and 800 ppm males and females were significantly lower than those of the controls. Mice exposed to 400 ppm or greater were lethargic and exhibited hunched posture and rough coats. Absolute and relative lung weights of 600 and 800 ppm males and the relative lung weight of 600 ppm females were significantly greater than those of the controls. Chemical-related hyperplasia of the bronchiolar epithelium was observed in all exposed groups of males and females. Lymphocytic atrophy of the spleen and thymus was observed in males and females exposed to 400 ppm or greater.

13-Week Study in Rats

Groups of 10 male and 10 female F344/N rats were exposed to 0, 10, 25, 75, 150, or 300 ppm (approximately 42, 105, 315, 630, or 1,260 mg/m^3) isobutyl nitrite by inhalation for 6 hours per day, 5 days per week for 13 weeks. All rats survived to the end of the study. Final mean body weights and mean body weight gains of 300 ppm males and females were significantly lower than those of the controls, as was the mean body weight gain of 150 ppm females. Clinical findings observed during the study included ruffled fur in 300 ppm males and females, hypoactivity in 300 ppm males, and hyperactivity in 150 and 300 ppm females. A very mild chemical-related methemoglobinemia and anemia occurred in male and female rats in the 75, 150, and 300 ppm groups. Hematopoietic hyperplasia occurred in the bone marrow of all exposed groups of males and females and was considered to be a secondary response to the anemia and methemoglobinemia. There was minimal hemosiderin pigment accumulation in the spleens of males and females exposed to 75 ppm or greater, mild to moderate epithelial cell hyperplasia of the nasal mucosa was observed in 300 ppm males and females, and minimal hyperplasia occurred in 150 ppm males and females. Hyperplasia of the bronchial epithelium was observed in 300 ppm males and females.

13-Week Study in Mice

Groups of 10 male and 10 female B6C3F$_1$ mice were exposed to 0, 10, 25, 75, 150, or 300 ppm (approximately 42, 105, 315, 630, or 1,260 mg/m^3) isobutyl nitrite by inhalation for 6 hours per day, 5 days per week for 13 weeks. There were no chemical-related deaths. Final mean body weights and mean body weight gains of 150 and 300 ppm females were significantly less than those of the controls. Final mean body weights and mean body weight gains of exposed groups of males were similar to those of the controls. There were no chemical-related clinical findings. A very mild chemical-related methemoglobinemia occurred in male and female mice in the 150 and 300 ppm groups. A very mild anemia occurred in the 300 ppm groups. In the lung, increased incidences of mild to moderate hyperplasia of the bronchiolar epithelium occurred in males and females exposed to 300 ppm. Minimal hyperplasia occurred in males exposed to 75 ppm or greater and in females exposed to 150 ppm. Minimal epithelial cell hyperplasia of the nasal mucosa was observed in 300 ppm males. Increased hematopoiesis of the spleen, secondary to the hematotoxicity, occurred in males exposed to 75 ppm or greater and in females exposed to 150 or 300 ppm. Increased hemosiderosis of the spleen occurred in males exposed to 300 ppm and in females exposed to 75 ppm or greater.

2-Year Study in Rats

Based on the low final mean body weights, anemia, and the mild to moderate nasal mucosal lesions and the hyperplastic bronchial lesions observed in 300 ppm males and females, isobutyl nitrite exposure concentrations selected for the 2-year inhalation study in rats were 37.5, 75, and 150 ppm.

Groups of 56 male and 56 female rats were exposed to 0, 37.5, 75, or 150 ppm (equivalent to 0, 158, 315, or 630 mg/m^3) isobutyl nitrite by inhalation for 6 hours per day, 5 days per week, for 103 weeks. Ten male and 10 female rats from each group were evaluated at 15 months for clinical pathology and histopathology.

Survival, Body Weights, Clinical Findings, Hematology, and Clinical Chemistry

Survival rates of exposed groups of rats were greater than those of the controls, and the survival rates of 75 and 150 ppm males were significantly greater than that of the control. Mean body weights of 150 ppm males and females were 3% to 11% lower than those of the controls throughout the course of the study. There were no clinical findings considered to be related to isobutyl nitrite exposure. A very mild methemoglobinemia and anemia occurred in male and female rats exposed to 75 or 150 ppm.

Pathology Findings

Incidences of alveolar/bronchiolar adenoma and alveolar/bronchiolar adenoma or carcinoma (combined) occurred with significant positive trends in exposed males and females, and the incidences of these neoplasms in 75 ppm males and in 150 ppm males and females were significantly greater than those in the controls. The incidence of alveolar/bronchiolar carcinoma was significantly greater in 150 ppm male rats than that in the controls. The incidences of alveolar epithelial hyperplasia were also increased in 75 and 150 ppm males and in all exposed groups of females. The incidences of mononuclear cell leukemia in exposed groups of males and females were significantly less than those in the controls.

2-YEAR STUDY IN MICE

Based on the low final mean body weight of 300 ppm females and the mild to moderate bronchiolar hyperplasia observed in 300 ppm males and females, isobutyl nitrite exposure concentrations selected for the 2-year inhalation study in mice were 37.5, 75, and 150 ppm.

Groups of 60 male and 60 female mice were exposed to 0, 37.5, 75, or 150 ppm (equivalent to 0, 158, 315, or 630 mg/m^3) isobutyl nitrite by inhalation for 6 hours per day, 5 days per week, for 103 weeks. As many as 10 male and 10 female mice from each group were evaluated at 15 months for clinical pathology and histopathology.

Survival, Body Weights, Clinical Findings, Hematology and Clinical Chemistry

Survival rates of exposed groups of males were similar to those of the controls. Survival rates of exposed groups of females were greater than those of the controls, and the survival rate of 37.5 ppm females was significantly greater than that of the controls. Mean body weights of exposed groups of males and of 37.5 and 75 ppm females were similar to those of the controls throughout the study. Mean body weights of 150 ppm females were lower than those of the controls from week 20 until the end of the study. There were no biologically significant clinical findings noted in the 2-year study in mice. A very mild methemoglobinemia and anemia occurred in male and female mice exposed to 75 or 150 ppm.

Pathology Findings

Incidences of alveolar/bronchiolar adenoma and alveolar/bronchiolar adenoma or carcinoma (combined) occurred with significant positive trends in exposed males and females, and the incidences of these neoplasms were significantly greater than those in the controls in 75 ppm males and in 150 ppm males and females. Incidences of alveolar epithelial hyperplasia were significantly increased in 75 and 150 ppm male and female mice. Thyroid gland follicular cell adenoma occurred with a significant positive trend in male mice; the incidences of thyroid gland follicular cell hyperplasia were increased in all exposed groups of males, and the incidences in males exposed to 37.5 or 150 ppm were significantly greater than those in the controls. Incidences of serous exudate and olfactory epithelium atrophy in the nose of 150 ppm females were significantly greater than those in the controls. Incidences of minimal to mild hemosiderin pigment in the spleen of 75 and 150 ppm male mice were significantly greater than those in the controls.

GENETIC TOXICOLOGY

Isobutyl nitrite was found to be mutagenic *in vitro* and *in vivo*. It induced base-pair substitution mutations in *Salmonella typhimurium* strains TA100 and

TA1535 and sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells. Positive responses in the *S. typhimurium* tests required S9 activation, but isobutyl nitrite induced chromosomal effects in cultured Chinese hamster ovary cells with and without S9. *In vivo*, no induction of sex-linked recessive lethal mutations was noted in the germ cells of male *Drosophila melanogaster* exposed to isobutyl nitrite via feeding or injection. However, significant increases in micronucleated normochromatic erythrocytes were observed in the peripheral blood of male and female mice treated with isobutyl nitrite for 90 days by inhalation.

CONCLUSIONS

Under the conditions of these 2-year inhalation studies, there was *clear evidence of carcinogenic activity** of isobutyl nitrite in male and female F344/N rats based on the increased incidences of alveolar/bronchiolar adenoma and alveolar/bronchiolar adenoma or carcinoma (combined). There was *some evidence of carcinogenic activity* of isobutyl nitrite in male and female $B6C3F_1$ mice based on the increased incidences of alveolar/bronchiolar adenoma and alveolar/bronchiolar adenoma or carcinoma (combined) in males and females. The increased incidence of thyroid gland follicular cell adenoma in male mice may have been related to isobutyl nitrite exposure.

Exposure of rats and mice to isobutyl nitrite by inhalation for 2 years resulted in increased incidences of alveolar epithelial hyperplasia (male and female rats and mice), thyroid gland follicular cell hyperplasia and splenic hemosiderin pigmentation (male mice), and serous exudate and atrophy of the olfactory epithelium of the nose (female mice).

Exposure of rats to isobutyl nitrite by inhalation for 2 years resulted in decreased incidences of mononuclear cell leukemia in males and females.

---

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 10. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 12.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Isobutyl Nitrite

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Doses	0, 37.5, 75, or 150 ppm isobutyl nitrite by inhalation	0, 37.5, 75, or 150 ppm isobutyl nitrite by inhalation	0, 37.5, 75, or 150 ppm isobutyl nitrite by inhalation	0, 37.5, 75, or 150 ppm isobutyl nitrite by inhalation
Body weights	150 ppm group slightly lower than controls	150 ppm group slightly lower than controls	Exposed groups similar to controls	150 ppm group lower than controls
2-Year survival rates	17/46, 23/46, 36/46, 28/46	29/46, 35/45, 31/46, 33/46	37/50, 35/50, 35/50, 30/53	32/51, 42/51, 36/50, 37/50
Nonneoplastic effects	Lung: alveolar epithelial hyperplasia (5/46, 8/46, 26/46, 31/46)	Lung: alveolar epithelial hyperplasia (3/46, 10/45, 11/46, 30/46)	Lung: alveolar epithelial hyperplasia (0/50, 4/50, 7/49, 13/53) Thyroid gland: follicular cell hyperplasia (8/50, 17/50, 12/50, 20/53) Spleen: hemosiderin pigmentation (28/50, 19/50, 46/49, 49/51)	Lung: alveolar epithelial hyperplasia (0/51, 2/51, 9/50, 8/50) Nose: serous exudate (1/51, 1/51, 2/50, 23/50); olfactory epithelial atrophy (0/51, 0/51, 1/50, 16/50)
Neoplastic effects	Lung: alveolar/ bronchiolar adenoma (0/46, 3/46, 12/46, 13/46); alveolar/ bronchiolar carcinoma (1/46, 2/46, 1/46, 6/46); alveolar/ bronchiolar adenoma or carcinoma (1/46, 5/46, 13/46, 15/46)	Lung: alveolar/ bronchiolar adenoma (0/46, 2/45, 2/46, 10/46); alveolar/ bronchiolar adenoma or carcinoma (0/46, 3/45, 2/46, 11/46)	Lung: alveolar/ bronchiolar adenoma (7/50, 12/50, 13/49, 17/53); alveolar/ bronchiolar adenoma or carcinoma (8/50, 16/50, 16/49, 19/53)	Lung: alveolar/ bronchiolar adenoma (4/51, 14/51, 7/50, 17/50); alveolar/ bronchiolar adenoma or carcinoma (6/51, 15/51, 9/50, 19/50)
Uncertain findings	None	None	Thyroid gland: follicular cell adenoma (1/50, 0/50, 0/50, 5/53)	None
Decreased incidences	Mononuclear cell leukemia: (27/46, 2/46, 1/46, 1/46)	Mononuclear cell leukemia: (14/46, 1/45, 0/46, 1/46)	None	None
Level of evidence of carcinogenic activity	Clear evidence	Clear evidence	Some evidence	Some evidence

Genetic toxicology

Salmonella typhimurium gene mutations:	Positive in strains TA100 and TA1535 with S9; negative in TA100 and TA1535 without S9; negative in TA98 and TA1537 with and without S9
Sister chromatid exchanges	
Cultured Chinese hamster ovary cells *in vitro*:	Positive with and without S9
Chromosomal aberrations	
Cultured Chinese hamster ovary cells *in vitro*:	Positive with and without S9
Sex-linked recessive lethal mutations	
Drosophila melanogaster:	Negative when administered by feed or injection
Micronucleated erythrocytes	
Mouse peripheral blood *in vivo*:	Positive in male and female mice

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on isobutyl nitrite on November 29, 1994, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

Arnold L. Brown, M.D., Chair
University of Wisconsin Medical School
Madison, WI

Thomas L. Goldsworthy, Ph.D., Principal Reviewer
Department of Experimental Pathology and Toxicology
Chemical Industry Institute of Toxicology
Research Triangle Park, NC

Meryl H. Karol, Ph.D., Principal Reviewer*
Department of Environmental Occupational Health
University of Pittsburgh
Pittsburgh, PA

Curtis D. Klaassen, Ph.D.
Department of Pharmacology and Toxicology
University of Kansas Medical Center
Kansas City, KS

Claudia S. Miller, M.D., M.S.
University of Texas Health Sciences Center
San Antonio, TX

Janardan K. Reddy, M.D.*
Department of Pathology
Northwestern University Medical School
Chicago, IL

Irma Russo, M.D.
Fox Chase Cancer Center
Philadelphia, PA

Louise Ryan, Ph.D.
Division of Biostatistics
Harvard School of Public Health and
Dana-Farber Cancer Institute
Boston, MA

Robert E. Taylor, M.D., Ph.D., Principal Reviewer
Department of Pharmacology
Howard University College of Medicine
Washington, DC

Mary Jo Vodicnik, Ph.D.*
Lilly MSG Development Center
Belgium

Jerrold M. Ward, D.V.M., Ph.D.*
National Cancer Institute
Frederick, MD

* Did not attend

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On November 29, 1994, the draft Technical Report on the toxicology and carcinogenesis studies of isobutyl nitrite received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. K.M. Abdo, NIEHS, introduced the toxicology and carcinogenesis studies of isobutyl nitrite by discussing the uses of the chemical and rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on the chemical-related neoplastic and nonneoplastic lesions in male and female rats and mice. The proposed conclusions were *clear evidence of carcinogenic activity* in male and female F344/N rats and *some evidence of carcinogenic activity* in male and female B6C3F$_1$ mice.

Dr. Taylor, a principal reviewer, agreed with the proposed conclusions. He commented that a statement in the Introduction should be amended to indicate that the only data in the literature on the carcinogenicity of isobutyl nitrite in humans were equivocal and came from an immunocompromised population. Dr. Abdo agreed (page 18).

Dr. Karol, the second principal reviewer, was unable to attend the meeting but had submitted her review, which Dr. L.G. Hart, NIEHS, read into the record. Dr. Karol agreed with the proposed conclusions. She stated that because the rationale for studying isobutyl nitrite was its possible contribution to the high incidence of Kaposi's sarcoma among male homosexuals with acquired immune deficiency syndrome (AIDS), a discussion of the relevance of the findings to the development of the lesions in AIDS patients should be added. Dr. Abdo acknowledged that the primary neoplastic lesions were in the lungs while Kaposi's sarcoma is a skin lesion, but he noted that it is not unusual for a chemical to have different target sites in different species. Dr. R.C. Sills, NIEHS, commented that laboratory rodents have no spontaneously occurring lesions morphologically similar to Kaposi's sarcoma in humans.

Dr. Goldsworthy, the third principal reviewer, agreed with the proposed conclusions although he thought that if further certainty could be obtained associating chemical exposure with increased thyroid follicular cell adenomas, the conclusion could be changed to clear evidence in male mice. Dr. Sills responded that these neoplasms were placed in the category of uncertain findings because there was no significant increase in the incidence of follicular cell carcinomas, no dose-response relationship for follicular cell adenomas, and no similar response in female mice. Dr. Goldsworthy commented on the differing purity of the four different lots of isobutyl nitrite and wondered if this could have affected the observed results. Dr. Abdo reported that the lots used for the 2-year studies were 97% to 99% pure and it was thought that the results were not affected by the level of contaminant present.

Dr. Miller asked whether there was information on the short-term concentrations that humans would experience in using the chemical, presumably from aerosol cans. Dr. Abdo said the labels on the cans did not give concentrations.

Dr. Taylor moved that the Technical Report on isobutyl nitrite be accepted with the revisions discussed and with the conclusions as written for male and female rats, *clear evidence of carcinogenic activity* in male and female F344/N rats and *some evidence of carcinogenic activity* for male and female B6C3F$_1$ mice. Dr. Russo seconded the motion, which was accepted unanimously with six votes.